Electrical coupling of isolated cardiomyocyte clusters grown on aligned conductive nanofibrous meshes for their synchronized beating

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Abstract

Myocardial infarction is often associated with abnormalities in electrical function due to a massive loss of functioning cardiomyocytes. This work develops a mesh, consisting of aligned composite nanofibers of polyaniline (PANI) and poly(lactic-co-glycolic acid) (PLGA), as an electrically active scaffold for coordinating the beatings of the cultured cardiomyocytes synchronously. Following doping by HC1, the electrospun fibers could be transformed into a conductive form carrying positive charges, which could then attract negatively charged adhesive proteins (i.e. fibronectin and laminin) and enhance cell adhesion. During incubation, the adhered cardiomyocytes became associated with each other and formed isolated cell clusters; the cells within each cluster elongated and aligned their morphology along the major axis of the fibrous mesh. After culture, expression of the gap-junction protein connexin 43 was clearly observed intercellularly in isolated clusters. All of the cardiomyocytes within each cluster beat synchronously, implying that the coupling between the cells was fully developed. Additionally, the beating rates among these isolated cell clusters could be synchronized via an electrical stimulation designed to imitate that generated in a native heart. Importantly, improving the impaired heart function depends on electrical coupling between the engrafted cells and the host myocardium to ensure their synchronized beating.

Keywords: myocardial infarction; cardiomyocyte; arrhythmia; conductive polymer; myocardial tissue engineering

1. Introduction

Myocardial infarction is often associated with abnormalities in the electrical function of the cardiovascular system known as arrhythmias, subsequently impairing cardiac performance severely. As is well known, massive loss of functioning cardiomyocytes is responsible for triggering cardiac arrhythmias [1,2]. Despite the effectiveness of cell-based therapies in repairing infarcted myocardial tissues, the lack of functional coupling of donor cells with the viable host tissues can significantly impede their electrical communications. To reestablish the contractile function of an infarcted heart, we hypothesize on the ability to use electrically active scaffolds in order to integrate implanted cells with the host myocardium in a synchronized manner. The materials used to fabricate electrically active scaffolds require matched excitability of grafted and host cells, as well as support propagation of the electrical wavefront.

This work develops an aligned nanofibrous mesh with electrically conducting properties as a tissue-engineered scaffold to provide structural support for neonatal rat cardiomyocytes. The mesh, consisting of composite nanofibers of polyaniline (PANI)/poly(lactic-co-glycolic acid) (PLGA), is fabricated by an electrospinning technique. Electrospinning offers ultrafine fibers, provides high exposure to a cell environment, and allows nutrient transport. Additionally, the aligned electrospun nanofibers have structures that resemble those of the natural extracellular matrices (ECMs) of cardiac tissues [3,4]. This aligned fibrous mesh can thus serve as a functional unit by providing the topographic cue for cell alignment. In the native heart tissue, alignment of cardiomyocytes contributes to the anisotropic (i.e. directionally dependent) tissue structure of the heart, thereby facilitating its coordinated electrical propagation and mechanical contraction [3,5,6].

PANI is one of the best-characterized conducting polymers, due to its ease of synthesis, diversity of structural forms and highly environmental stability [7]. A π-conjugated backbone

within PANI allows it to conduct electrons, providing PANI with the opportunity to couple grafted cells with the host tissue electrically. The PANI polymer has been used for biomedical applications both *in vitro* and *in vivo* [8–11].

The solubility of PANI in most common organic solvents is unsatisfactory, making it difficult to fabricate a uniform structure of electrospun nanofibers with PANI independently. Therefore, the aligned fibrous mesh is fabricated using a blend solution of PANI/PLGA for electrospinning. Owing to its excellent biodegradability, PLGA has received considerable attention as scaffold materials for tissue engineering [12–14].

This work elucidates the fundamental material properties of the fabricated fibrous meshes, including their morphology, surface characteristics, electrical conductivity, and cell compatibility. Neonatal rat cardiomyocytes on test fibrous meshes are also cultivated, along with their beating behaviors examined before and after electrical stimulation (Fig. 1). Moreover, synchronous contractions of the cultivated cardiomyocytes are induced by applying electrical signals designed to mimic those in the native heart.

2. Materials and Methods

2.1. Materials

PLGA (LA/GA = 75/25, MW = 66–107 kDa), 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), PANI emeraldine base (PANI-EB) of MW 65 kDa, and hydrochloric acid (HCl) were purchased from Sigma-Aldrich (St Louis, MO, USA). Cell culture reagents were obtained from Invitrogen (Carlsbad, CA, USA).

2.2. Preparation and characterization of aligned nanofibrous meshes

PANI-EB was dissolved in HFIP (0.5% by w/v), and stirring was continued overnight. This PANI-EB solution was filtered through a 0.45 μm filter unit before adding PLGA (6%, 8%, 10%, or 12% by w/v). Both PANI and PLGA are soluble to some extent in HFIP [15,16].

HFIP is known to be cytotoxic. However, its boiling point is only 59℃ [17] and can be easily evaporated; therefore, HFIP has been commonly used as a solvent for electrospinning [3,16–18].

The mixed solution was loaded into a 1-mL syringe and delivered at a constant flow rate (1 mL/h) through a stainless steel needle (23 gauge) connected to a high-voltage power supply. Once a high voltage (12.3–13.6 kV) was applied, a thin polymer solution jet was ejected from the needle and deposited on a thick aluminum foil wrapped around a custom-made high-speed rotating mandrel (Fig. 1). After HFIP was evaporated at room temperature overnight, an aligned PANI/PLGA mesh was obtained. 1*N* HCl was used as a dopant to increase the conductivity of the as-prepared PANI/PLGA mesh, by converting the insulating PANI-EB into a conductive form (PANI emeraldine salt, PANI-ES) through protonation of the polymer overnight [19].

Morphology of the electrospun nanofibrous meshes was examined using a scanning electron microscope (SEM, Model JSM-5600, JEOL, Tokyo, Japan). Based on the obtained SEM images, the diameters and angle distributions of their constituted nanofibers were then evaluated using an analysis software (Image-Pro Plus, Media Cybernetics, Silver Spring, MD, USA) [16]. Next, the PANI/PLGA meshes were characterized by Fourier-transform infrared (FT-IR) spectroscopy (Perkin-Elmer, Buckinghamshire, UK) and X-ray photoelectron spectroscopy (XPS, ESCA PHI 1600, Physical Electronics, Chanhassen, MN, USA). Additionally, their conductivity was measured by using a 4-probe technique (Quatek 5601Y/QT-50, Taiwan). The mechanical properties of test meshes were measured using an Instron 5543 mechanical testing instrument (Instron Corp., Norwood, MA, USA).

Their protein adhesive properties were then investigated by soaking the undoped and doped PANI/PLGA meshes in the horse serum (Gibco, Grand Island, NY, USA) for 1 h and then washing them 3 times with deionized (DI) water. Subsequently, the meshes were stained

with rabbit anti-laminin and mouse anti-fibronectin (Abcam, Cambridge, MA, USA). Additionally, fluorescent colors were obtained using different Alexa-Fluor secondary antibodies (Invitrogen). Finally, the stained meshes were examined using an inverted confocal laser-scanning microscope (CLSM, TCS SL, Leica, Germany).

2.3. Cardiomyocyte isolation and cell culture

Neonatal cardiomyocytes were isolated from the hearts of 1–2-day-old Lewis rats using a Worthington Neonatal Cardiomyocyte Isolation System (Worthington Biochemical, Lakewood, NJ, USA). Prior to cell seeding, test PANI/PLGA fibrous meshes were cut into small squares with an area of 1.5 × 1.5 cm^2, sterilized with UV light overnight, and pre-incubated for 1 h in the horse serum at 37℃. The isolated cardiomyocytes were seeded onto test meshes at a density of 5 × 10^4 cells/cm^2 and then cultured in the growth medium (Dulbecco's modified Eagle's medium, Gibco) supplemented with 5% chick embryo extract (Sera Laboratories, West Sussex, United Kingdom), 10% horse serum, 100 U/mL penicillin, and 100 μg/mL streptomycin (Invitrogen).

In the electrical stimulation experiment, cells were labeled with a lipophilic dye (1,1′-dioctadecyl-3,3,3′,3′-tetra-methylindo-carbocyanine perchlorate, Dil) before seeding. Briefly, the isolated cardiomyocytes were incubated in a 5μM Dil solution at 37℃ for 15 min. After the dye diffused laterally within the cellular plasma membranes, the supernatant was removed via centrifugation at 1000 rpm, and the cells were gently resuspended in a pre-warmed (37℃) growth medium and then seeded on test meshes.

2.4. Cell viability tests

The viability of cardiomyocytes on test meshes was evaluated using a Live/Dead Viability Kit (Invitrogen) and photographed by a fluorescence microscope (Axio Observer Z1, Zeiss, Göttingen, Germany). As an additional test, the leakage of lactate dehydrogenase (LDH) in the culture media was measured. After cell culturing for distinct periods, media

were collected and centrifuged; in addition, the activity of LDH released from the cytosol of damaged cells was assessed using the LDH Cytotoxicity Assay Kit (Sigma-Aldrich). The maximal LDH release of cells was determined by adding a lysis solution (0.8% Triton X-100), followed by incubation at 37℃ for 45 min [20]. Finally, the optical density at a wavelength of 490 nm was determined using a multiwell scanning spectrophotometer (Dynatech Laboratories, Chantilly, VA, USA).

2.5. Immunostaining of cardiomyocytes

After 60 h of culture, the cells on test meshes were fixed for immunofluorescent staining. The antibodies used were mouse anti-cardiac Troponin I and rabbit anti-connexin 43 (Abcam). Colored fluorescence was then generated using various Alexa-Fluor secondary antibodies. Finally, cells were costained to visualize the nuclei by propidium iodide (Sigma-Aldrich) and then examined using fluorescence microscopy.

2.6. Electrical stimulation

In this work, test PANI/PLGA meshes were placed on a glass slide. Two silver wires were placed under two ends of the mesh with the direction perpendicular to the major axis of the fibers (Fig. 1) [18]. The product was subsequently covered by a glass well (1 cm × 1 cm inner well dimension) that acted as a sealant and prevented the wires from coming into direct contact with the medium. This assembly was tightly sealed with a silicone paste (Dow Corning Corp., Midland, MI, USA) and sterilized by exposure to UV light overnight.

The Dil-labeled cardiomyocytes were then seeded on test meshes; following culturing, trains of electrical pulses (1.25 Hz, 5 V/cm) were applied, which is characteristic of native myocardium [21]. The beating behaviors of the cardiomyocytes (before and after electrical stimulation) were then observed and recorded using a fluorescence microscope with a CCD camera. The video was recorded at 30 fps and in a resolution of 640 × 480 pixels. The images were digitized frame by frame with Image-Pro Plus software. Finally, the beating rate of the

culture was analyzed by selecting and tracking a small area of the video frame sequence in a cell cluster where brightness clearly oscillated due to the cell beating [22].

2.7. Statistical analysis

Two groups were compared by the one-tailed Student's *t*-test using statistical software (SPSS, Chicago, IL, USA). Data are presented as mean ± SD. A difference of $P < 0.05$ was considered statistically significant.

3. Results and Discussion

After myocardial infarction, electrical integrity of the heart is often compromised because of massive loss of functioning cardiomyocytes. By combining cells and scaffolds, cardiac tissue engineering is a promising means of treating patients suffering from myocardial infarction [23–25]. One of the challenges for cardiac tissue engineering is that the implanted donor cells should be coupled electrically to the local host tissue [26,27]. The lack of electrical coupling of donor cells with the viable host myocardium significantly impedes cell-to-cell signaling, leading to unsynchronized beating between the tissue-engineered cardiac patch and the host tissue [26,28,29]. This work demonstrates the feasibility of using an aligned PANI/PLGA nanofibrous mesh as an electrically active scaffold to coordinate synchronous beating of grown isolated cardiomyocyte clusters.

3.1. Morphology of test nanofibrous meshes

A viscous solution was prepared to fabricate the aligned fibrous meshes by blending PANI-EB with PLGA in HFIP. Figure 2a illustrates the SEM micrographs of the electrospun fibrous meshes prepared at distinct weight ratios of PANI/PLGA (by w/v). At a PANI/PLGA ratio of 0.5%/6%, although continuous, the electrospun fibers were not aligned properly with bead defects; reducing the PLGA concentration further induced the formation of beads significantly. Additionally, aligned ultrafine fibers in a structure of porous meshes without

the occurrence of bead defects were obtained by increasing the weight ratio of PLGA used (0.5%/8%, 0.5%/10%, and 0.5%/12%). Moreover, a higher concentration of PLGA used implied a thicker diameter of the electrospun fibers (0.5%/6%: 58.9 ± 14.2 nm; 0.5%/8%: 101.7 ± 12.7 nm; 0.5%/10%: 140.3 ± 14.8 nm; and 0.5%/12%: 184.7 ± 31.9 nm).

Next, the alignment of electrospun fibers was characterized by performing statistical analysis of the angles between the fibers and their major axis. Figure 2b reveals that the angle distribution fluctuated in a wide range of 0°–90° for the mesh prepared at a low concentration of PLGA (i.e. PANI/PLGA = 0.5%/6%). Increasing the PLGA concentration (0.5%/8%, 0.5%/10%, or 0.5%/12%) significantly narrowed the angles of most fibers from 0°–10°, indicating that nearly all fibers were oriented along the major axis of the meshes, with a unique aligned topography.

Here, HCl was used as a dopant, allowing the protonation of the =N– atoms on PANI-EB, to increase the conductivity of the electrospun fibers. After doping, the structure of fibrous meshes did not appear to be damaged (Fig. 2a). However, the color of the meshes changed from blue to green (Fig. 1). As is well known, the doping process alters the PANI electronic structure, subsequently producing new electronic states in the band gap and ultimately causing color changes [30].

3.2. Chemical characteristics of PANI/PLGA nanofibrous meshes

The chemical characteristics of the electrospun fibers were determined by FT-IR and XPS spectroscopies. Figure 3 shows the FT-IR spectra of pure PANI, PLGA, and the electrospun fibers before and after doping. According to this figure, the characteristic peaks of pure PLGA (C=O stretching ester, 1755 cm^{-1}) and PANI (C=C stretching vibrations of diiminoquinoid and benzenoid rings at 1588 cm^{-1} and 1495 cm^{-1}, respectively) were appeared in the spectrum acquired from the electrospun fibers [31,32], implying the presence of PANI and PLGA. After doping by HCl, the PANI characteristic peaks of quinoid and

benzenoid rings at 1588 cm^{-1} and 1495 cm^{-1} were, respectively, red-shifted to 1576 cm^{-1} and 1489 cm^{-1}, due to a significant increase in molecular dipole moment. This finding suggests that the electrospun fibers were in their conductive state carrying positive charges ($=N^{+}H–$) [33,34]. Restated, PANI transformed from an insulator (PANI-EB form) to a conductor (PANI-ES form).

Whether the imino units (i.e. =N–) on the electrospun fibers were protonated was further investigated by performing an XPS study. Figure 4 illustrates the N1s core-level spectra of the electrospun fibers before and after doping. A single peak was observed in the spectrum of the undoped fibers, and could be curve-fitted to two components with binding energies at 398 eV (imine nitrogen, =N–) and 399 eV (amine nitrogen, –NH–). After doping by HCl, the line shape of the N1s spectrum changed and could be further decomposed into three major peaks with binding energies at 398 eV, 399 eV, and at 401 eV (i.e. a newly generated peak corresponding to the cationic nitrogen atoms, $–N^{\bullet+}H–$) [35,36]. Above results suggest that the as-prepared electrospun fibers were in their neutral form. Following HCl doping, the fibers could be transformed into a conductive form carrying positive charges.

3.3. Conductivity of PANI/PLGA fibrous meshes

Various dopants have been used to transform the neutral form of PANI-EB into the conductive form of PANI-ES [19]. Oxidative dopants such as I_2, $FeCl_3$, and AsF_5 are known to be cytotoxic [37]. On the other hand, the protonic acid doping procedure using HCl could significantly increase the conductivity of PANI by a 9 to 10 order of magnitude [38]. Consequently, HCl was chosen as a doping agent in the study.

The dopant produces new energy levels between the conduction and valence bands, subsequently rendering metallic conductivity [39]. According to Fig. 5a, the fibrous mesh prepared at a PANI/PLGA ratio of 0.5%/12% revealed a rather poor conductivity (9.5×10^{-7} S/cm). Notably, increasing the relative proportion of PANI used in electrospinning (i.e.

decreasing the PLGA concentration) significantly improved the conductivity of the obtained fibrous meshes ($P < 0.05$). For example, the test mesh prepared at a PANI/PLGA ratio of 0.5%/8% gained more than three orders of magnitude in conductivity (3.1×10^{-3} S/cm). Further increasing in relative PANI proportion would not significantly increase its conductivity ($P > 0.05$), possibly owing to bead defects in the mesh, as discussed above (Fig. 2a).

However, it was observed that test meshes, maintained in the culture medium, retained a significant level of electrical conductivity for at least 100 h, although this conductivity decreased gradually over time due to the deprotonation of PANI [40]. Based on the above experimental results, the mesh prepared at a PANI/PLGA ratio of 0.5%/8% was selected, which possessed an aligned fibrous structure (Fig. 2a) and the highest conductivity among all of the test groups, for the following studies. The Young's modulus of such a mesh (91.7 ± 5.1 MPa, $n = 5$) was in the order of magnitude of the PLLA/PANI nanofibrous mesh reported in literature [41].

3.4. Protein adsorption and cell adhesion, viability and alignment on fibrous meshes

The surface properties of substrates (e.g. the surface charge) markedly affect the protein adsorption, thereby governing their subsequent cell adhesion and viability [42,43]. Cell adhesion to surfaces is mediated by a layer of adsorbed proteins, such as fibronectin and laminin, which are negatively charged when present in the physiological conditions [44,45]. As mentioned earlier, HCl doping transformed the fibrous meshes from neutral into positively charged ones, possibly attracting the negatively charged fibronectin and laminin [46]. According to Figs. 5b and 5c, fractions of the surface of the undoped mesh (neutral) adsorbed fibronectin and laminin were significantly smaller than their doped counterparts (positively charged, $P < 0.05$). Consequently, poor cell adhesion was observed on the undoped mesh ($9.0 \pm 2.1 \times 10^3$ cells/cm^2), whereas the adsorption with fibronectin and

laminin on the doped surface enhanced cell adhesion and spreading ($4.7 \pm 0.2 \times 10^4$ cells/cm^2, Fig. 6a).

The viability of the neonatal rat cardiomyocyts cultivated on test meshes was evaluated qualitatively using a live/dead assay based on calcein-AM and ethidium homodimer and determined quantitatively by the LDH assay; the cells cultured on regular 6-well plates were used as a control. The hydrolysis of calcein-AM in live cells produces green fluorescence, while ethidium homodimer is excluded from entering live cells and only produces red fluorescence in dead cells [13]. The LDH assay is an effective means of measuring the membrane integrity (or cell viability) as a function of the amount of cytoplasmic LDH leaked into the medium, expressed as the percentage of total LDH activity [47].

Most cells must attach and spread out on the substrate before they can start to thrive and proliferate; normal cells often interpret the lack of adhesion as a signal to commit suicide [48]. As cell adhesion on the undoped mesh was poor, the cell viability decreased appreciably with time (i.e. the LDH leakage increased significantly, $P < 0.05$, Fig. 6b). In contrast, the viability of cells grown on the doped mesh was comparable to the control group throughout the entire study ($P > 0.05$).

To be functionally efficacious, the engrafted cardiomyocytes must be aligned appropriately with the long axis of cardiac muscle fibers [49]. Electrospun oriented fibers can provide contact guidance to cultivated cells, leading to an alignment of the cells [5]. According to Fig. 6a, cardiomyocytes cultured on the doped fibrous mesh elongated and aligned their morphology along the major axis of the fibers. Conversely, such phenomenon was not observed on the surface of the undoped mesh, due to its poor cell adhesion as mentioned above. In contractile tissues such as myocardium, functional properties are directly related to the cellular elongation and orientation [50].

3.5. Immunostaining of cardiomyocytes

Electrical communication between cardiomyocytes occurs via gap junctions, which are composed of connexin proteins that mediate intercellular communication [51]. During incubation, our results indicated that the seeded cardiomyocytes became associated with each other and formed isolated cell clusters on the doped fibrous mesh (Fig. 7); similar results were also observed in the regular plates. Cardiomyocytes are considered terminally differentiated and incapable of proliferation [52]. Therefore, the isolated cell clusters cultured on test meshes could not reach confluence throughout the entire course of the study.

After culture, expression of cardiac-specific proteins such as cardiac troponin I (cTnI) and connexin 43 (Cx 43) was examined by immunostaining. The cells in isolated clusters expressed cTnI, a cardiac regulatory protein related to the contractile behavior of the heart tissue [53]. Additionally, expression of Cx 43 was clearly observed intercellularly in cell clusters. As a constitutive protein for the formation of cardiac gap junctions, Cx 43 is essential for both cell-cell coupling and the re-construction of functional tissues [27,52]. All of the cardiomyocytes in each cluster beat synchronously (Supplementary Video 1), suggesting that the coupling between cells was fully developed at this time. As is well established, myocardial tissues require a high cell density to ensure synchronous beating through gap junctions that tightly interconnect neighboring cells [54]. However, individual clusters beat at different rates, due to the lack of Cx 43 in between. Moreover, insufficient intercellular electrical coupling blocks the signal transfer among isolated clusters [1].

3.6. Electrical stimulation

Beating rates among these isolated clusters can be synchronized via an electrical stimulation designed to replicate that generated in the native heart [21]. Their beating rhythms were examined using high-speed photography images of individual clusters grown on test meshes [with a low (9.5×10^{-7} S/cm, PANI/PLGA = 0.5%/12%) or high conductivity (3.1×10^{-3} S/cm, PANI/PLGA = 0.5%/8%)]. As shown in Fig. 8 and Supplementary Video 2,

the isolated clusters cultured on both the low- and high-conductivity meshes beat asynchronously before electrical stimulation. After electrical stimulation, although relatively improved, beating frequencies for the individual clusters grown on the low-conductivity mesh remained unsynchronized. Conversely, contractions of the individual clusters grown on the high-conductivity mesh were synchronized, and the frequency of beating of the cell clusters was the same as that of oscillation of the electrical potential. It has been well established that the beating of cardiomyocytes is triggered by a transient increase in cytosolic free calcium, which occurs in response to depolarization of the cell membrane produced by the electrical excitation [55].

Above results suggest that the synchronous beating within each cluster is mediated by the presence of intercellular gap-junction protrin Cx 43, while the electrical field generated on the conductive mesh plays a significant role in coordinating the contraction between the isolated cell clusters (Fig. 1). Nevertheless, the culture medium may contribute conductivity, to some extent, due to its salt concentration.

4. Conclusions

An aligned conductive nanofibrous mesh of PANI/PLGA was successfully prepared using an electrospinning technique. After doping by HCl, the mesh could be transformed into a conductive form carrying positive charges, which is essential for the following cardiomyocyte adhesion, formation of isolated cell clusters, and electrical stimulation. The synchronous cell beating within each cluster is mediated by the intercellular gap-junction protein Cx 43, while the contractions of isolated clusters can be coupled together via an electrical stimulation. Successful myocardial tissue engineering requires the establishment of electric integration in the engineered graft to allow for synchronized beating.

Acknowledgements

This work was supported by grants from the National Science Council (NSC 98-2320-B-075A-001-MY3 and NSC 99-2628-B-075A-001-MY3), Taiwan (ROC).

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Figure Captions

Figure 1. Schematic diagrams displaying the processes for the fabrication of aligned conductive polyaniline (PANI)/ poly(lactic-co-glycolic acid) (PLGA) nanofibrous mesh, cell seeding, electrical stimulation and the mechanisms of the synchronous cell beatings.

Figure 2. (a) Scanning electron microscopy (SEM) images of the electrospun fibrous meshes prepared at distinct weight ratios of PANI/PLGA (by w/v); (b) their histograms of the fiber angle distribution.

Figure 3. FT-IR spectra of PANI, PLGA, and the electrospun fibrous meshes before and after doping.

Figure 4. XPS N1s core-level spectra of the electrospun fibrous meshes before and after doping.

Figure 5. (a) Conductivities of the electrospun fibrous meshes prepared at distinct PANI/PLGA ratios (by w/v); (b) confocal images of the fibronectin (red) and laminin (cyan) adhered on the undoped and doped meshes; (c) amounts of fibronectin and laminin adsorbed on the undoped and doped meshes expressed as normalized area fractions. *statistical significance at a level of $P < 0.05$; N.S.: not significant.

Figure 6. (a) Results of the live/dead assay of neonatal rat cardiomyocytes grown on test meshes at day 3; the cells cultured on regular 6-well plates were used as a control. Live cells were stained in green by calcein and dead cells were stained in red by ethidium; (b) results of the viability of neonatal rat cardiomyocytes cultivated on test meshes for distinct periods, quantitatively determined by the LDH assay. *statistical significance at a level of $P < 0.05$; N.S.: not significant.

Figure 7. Fluorescence micrographs of the neonatal rat cardiomyocytes cultivated on test meshes using the immunofluorescence staining for cardiac troponin I (cTnI, green), connexin 43 (Cx 43, blue), and nucleus (red). The inset in the panel shows a higher magnification image.

Figure 8. Results of the beating frequencies of isolated cell clusters (for the two shown in the images indicated by the red and blue arrows) grown on the low- and high-conductivity meshes (PNAI/PLGA = 0.5%/12% and 0.5%/8%, respectively) before and after electrical stimulation; their beating frequencies were analyzed by an imaging software and indicated by the red and blue lines, respectively, and the electrical potential frequency was denoted by the yellow line (the one with the yellow lightning symbol).

Supplementary Video Data

Video 1. The beating behaviors of the cells within each cluster; all of the cardiomyocytes in the cluster beat synchronously.

Video 2. The beating behaviors of the isolated cell clusters grown on the low- and high-conductivity meshes before and after electrical stimulation. The yellow lightning symbol represents the frequency of the stimulated electrical potential.